ISOELECTRIC POINTS OF RIBONUCLEOPROTEINS FROM TRANSPLANTABLE LINES OF RETICULAR CELLS SENSITIVE AND RESISTANT TO THE ACTION OF ENTEROVIRUSES

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In V. D. Solov'ev's laboratory transplantable monolayer cultures of cells of reticular type have been obtained, which retained their resistance through 30-60 passages to the cytopathogenic action of certain enteroviruses [1, 2]. The biological nature of the resistance of these cells to the viruses is unexplained.

The object of the present investigation was to compare the isoelectric points (IEP) of the ribonucleoproteins of cell lines resistant and sensitive to particular enteroviruses. This study was based on results obtained by A. L. Shabadash [3, 5], who showed that determination of the IEP by means of methylene blue in different pH zones can be used not only to discover in which concrete microstructures the ribonucleoproteins are located, but also to reveal physico-chemical differences between the ribonucleoproteins of the cell organoids.

EXPERIMENTAL METHOD

The investigation was carried out on two original cell lines sensitive to all enteroviruses: leukemia J-96 cells obtained from human blood [6] and MIO cells obtained from the tonsils of a monkey [1]. The comparison was drawn between the original J-96 cells and strain L-41, highly resistant to Coxsackie B3 virus, and also between the original MIO cells and strain MIO-45, relatively resistant to poliomyelitis virus.

The cells were grown in tubes in medium No. 199 on cover slips. Fixation was carried out by Shabadash's method. The IEP was shown histochemically by selective adsorption of methylene blue by Shabadash's method. For each experiment the color reaction was carried out on a series of fixed cells in 8 solutions with different pH values. Acetate and citrate buffer solutions with pH 2.6, 3.5, 4.2, 5.2, 6.2, 7.2, 8.0, and 9.2 were used (the pH was determined with a type LP-5 potentiometer).

EXPERIMENTAL RESULTS

The study of the nucleoli of the cultivated cells revealed five types of ribonucleoproteins differing in their isoelectric zones. This was in general agreement with results obtained by A. L. Shabadash [5], but the pH values at which the IEP of the nucleolar ribonucleoproteins were detected were not identical. The conclusions drawn by Shabadash regarding the five fractions of nucleolar ribonucleoproteins evidently reflected certain general biological principles, for these fractions were found in cells differing considerably in their functional and morphological characteristics.

The study of the IEP of the ribonucleoproteins of cells in different periods of cultivation showed that during aging of the cell cultures the nucleus, nucleolus, and perinucleolar chromatin (see figure, a) stained on the 2nd-3rd day at relatively more alkaline pH values than on the 5th-7th day. For example, in the MIO cells the nucleus and perinucleolar chromatin stained on the 2nd-3rd day at pH 4.2, but on the 5th-7th day at pH 3.5. In the MIO-45 cells these same cells structures stained on the 2nd-3rd day at pH 5.2 and on the 5th-7th day at pH 4.2.

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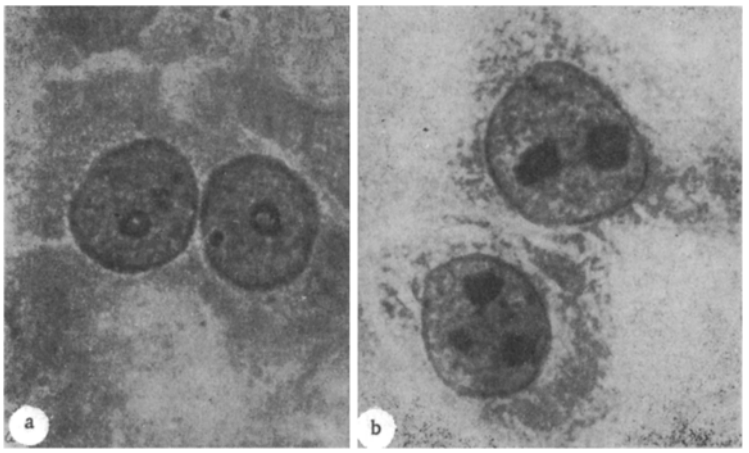

MIO cells. 3rd day. a) pH 4.2 (nucleus and perinucleolar chromatin stained); b) pH 6.2 (mitochrondria are clearly distinguishable against the general background of the cytoplasm). 2000×.

In the case of a relatively acid pH (2.6), neither the original nor the resistant cells stained appreciably; only a very weak diffuse staining of the nucleus could be detected. With increasing alkalinity the cell granules, which according to Shabadash [4] correspond to the ribonucleoprotein skeleton of the mitochondria, began to stain.

The sensitive J-96 cells did not take up the dye at pH 2.6. At pH 3.5 weak staining of the mitochondria, the nuclear chromatin, the perinucleolar chromatin, and the nucleolus was observed. At relatively alkaline pH values (5.2) they reacted intensively with the cations of the dye and were clearly distinguishable against the background of the cytoplasm. In the resistant L-41 cells the analogous components stained at pH 4.2.

In the MIO and MIO-45 cultures the cell components began to take up the dye at different pH values. In the original MIO cells the nucleus and the perinucleolar chromatin stained at pH 4.2, and became bright blue in color at pH 6.2-7.2. In the resistant MIO-45 cells the same cell components began to stain only at pH 5.2 (see Figure, b). At ph 7.2 they stained bright blue. The nucleoli also stained at different pH values. The staining first appeared as a greenish, amorphous mass (pH 2.6-4.2), becoming light blue in color at pH 5.2 in the sensitive cells and at pH 6.2 in the resistant cells.

There are thus grounds for supposing that the alkaline shift of the IEP is a special manifestation of the protective mechanisms of the cell, possibly associated with repression of particular areas of the cell DNA, leading to exclusion of certain components from the metabolism of the resistant cells essential for the development of the particular virus.

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